Received: 24 June 2019 | Accepted: 16 January 2020 | Published: 21 February 2020
DOI:10.20870/oeno-one.2020.54.1.2505

The impact of cluster thinning and leaf removal timing on the grape quality and concentration of monomeric anthocyanins in Cabernet-Sauvignon and Probus (*Vitis vinifera* L.) wines

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ABSTRACT

Aim: Leaf removal around clusters and cluster thinning are techniques usually applied in cool-climate vineyards in order to achieve optimal grape maturity. However, the impact of the timing of these two operations differs across varieties. Thus, the aim of the present work was to investigate the effects of cluster thinning and leaf removal timing (performed at three specific time points) on grape quality and monomeric anthocyanins in the wines of Cabernet-Sauvignon and Probus (Kadarka × Cabernet-Sauvignon, *Vitis vinifera* L.)

Methods and results: The experiment was conducted in Sremski Karlovci (Northern Serbia) in 2014, 2015, and 2016. Leaf removal was applied on six basal nodes of each shoot at three time points, 7 days after flowering, 30 days after flowering, and at veraison, i.e., at the onset of berry ripening. After cluster thinning, which was performed 7 days after flowering, one cluster per shoot was retained. On the treated vines, leaf removal treatment and cluster thinning were applied only once. Leaf removal was more effective than cluster thinning in respect to grape quality. Leaf removal, applied 7 and 30 days after flowering, decreased titratable acidity in Cabernet-Sauvignon, while in Probus an interaction of leaf removal and year was observed. Moreover, early leaf removal decreased the incidence of *Botrytis* sp. in Probus. The varieties reacted differently to cluster thinning in respect to grape quality: cluster thinning increased total soluble solids in Probus and lowered titratable acidity in Cabernet-Sauvignon. In 2015, both cluster thinning and leaf removal yielded changes in the anthocyanin ratios in the wines. Cluster thinning increased total and acylated anthocyanins in the wine of Cabernet-Sauvignon compared to wine derived from unthinned vines. The peonidin content was 40 % higher in the Cabernet-Sauvignon wine if the vines were subjected to leaf removal treatments.

Conclusions: Cluster thinning and leaf removal affected both Cabernet-Sauvignon and Probus (*Vitis vinifera* L.) grape quality **and** wine composition. Early leaf removal was the most effective treatment in both varieties. Therefore, combined application of cluster thinning and early leaf removal is highly recommended in the production of high-quality red wines in Serbia.

Significance and impact of the study: Timing of leaf removal application was usually investigated around flowering and veraison. Our results suggested that leaf removal between these two phenological stages also improves grape quality and changes the ratio of the monomeric anthocyanins in the wine.

KEYWORDS

leaf removal, cluster thinning, Cabernet-Sauvignon, Probus, quality

Supplementary data can be downloaded through: https://oeno-one.eu/article/view/2505

INTRODUCTION

In most of the wine regions worldwide, the production of high-quality wine is a challenge. Among many environmental factors, climate has the greatest impact on vine development and grape quality. Wine-producing regions are characterised by mean climatic conditions, which are major drivers of wine quality in relation to its origin (van Leeuwen and Dariet, 2016). However, even in a given wine region these conditions vary from year to year.

In addition to climate, grape quality depends on the grape variety and viticulture practices. The viticulture practices of cluster thinning (CT) and leaf removal (LR) are commonly performed to improve grape quality.

CT can increase total soluble solid concentration (TSS) (Reynolds *et al.*, 1994; Valdes *et al.*, 2009) and pH (Valdes *et al.*, 2009) of the grape juice. It can also speed up ripening (Barros *et al.*, 2018), which could be useful especially in regions with unfavourable conditions during grape ripening. This technique also increases ethylene production in some fruits, indicating advance maturity (Lopez *et al.*, 2011). However, in other trials limited or no effects of CT on the grape quality were shown (Ough and Nagaoka, 1984; Keller *et al.*, 2005).

LR in the fruit zone is one of the most important and commonly applied canopy management operations in viticulture. This technique is performed on grapevines to improve light penetration and air circulation around the clusters. It is also applied to increase penetration of fungicide sprays and decrease disease incidence. LR can lead to increased levels of TSS (Bledsoe, 1988; Intrieri *et al.*, 2008; Kemp, 2010), total anthocyanins (Tardaguila *et al.*, 2010; Drenjančević *et al.*, 2017), and decreased titratable acidity (TA) (Petrie *et al.*, 2003). However, different results can be observed depending on the climate, variety and time of application.

The right moment for LR varies depending on the region, variety, and type of wine produced. In the past, it was usually performed around veraison (onset of ripening). Aćimović *et al.* (2016) found that removal of fewer than six leaves did not significantly affect the final yield per vine and some grape quality parameters.

Recently, positive a effect of early LR (around flowering) on the grape quality was observed (Moreno *et al.*, 2017). Early LR significantly decreases fruit set, which in turn increases cluster looseness and tolerance to rot (Poni *et al.* 2006; Diago *et al.*, 2010). Also, early LR can significantly decrease yield (Tardaguila *et al.*, 2010) and cluster weight (Petrie *et al.*, 2003; Intrieri *et al.*, 2008). One of the most positive effects of LR is reducing the incidence of *Botrytis cinerea* (Sivilotti *et al.*, 2016).

The varieties react differently to CT and time of LR. Moreover, there is a lack of knowledge on how LR applied at other times during berry development in combination with previously applied CT will affect the grape and wine quality.

The aim of this study was to investigate the effects of CT (performed 7 days after flowering) and timing of LR (7 days after flowering, 30 days after flowering, at veraison) on grape and wine quality parameters of Cabernet-Sauvignon and Probus (Kadarka × Cabernet-Sauvignon, *Vitis vinifera* L.).

MATERIALS AND METHODS

The experiment was conducted over a 3-year period (2014−2016) at the experimental field of the University of Novi Sad, Faculty of Agriculture, situated in Sremski Karlovci − Fruska Gora (45º10’ N, 20º10’ E). Fruska Gora is one of the most important Serbian wine-growing districts and is located in the Srem region. Cabernet-Sauvignon and Probus (VIVC variety number 9719) vines, grafted on SO4 rootstock, were planted in 2000, in a northeast-southwest orientation with 2.8 m spacing between rows and 1.6 m separation between pair of vines in a row. Vines were vertical shoot positioned (VSP) Guyot pruned with one cane and one spur (14 buds per vine). LR was applied on six basal nodes of each shoot. After CT, which was performed 7 days after flowering, one cluster per shoot remained.

Eight treatments were compared in the present study (Figure 1), four of which did not involve CT: (1) ED - LR performed 7 days after flowering; (2) MD - LR performed 30 days after flowering; (3) LD - LR performed at veraison, and (4) UN - no LR was performed. The remaining four treatments involved the same LR strategies as above, but also included cluster thinning: (5) ED+CT - both LR and cluster thinning were performed 7 days after flowering; (6) MD+CT - LR performed 30 days after flowering, with cluster thinning applied 7 days after flowering; (7) LD+CT - LR performed at veraison, while cluster thinning was applied 7 days after flowering; and (8) UN+CT - cluster

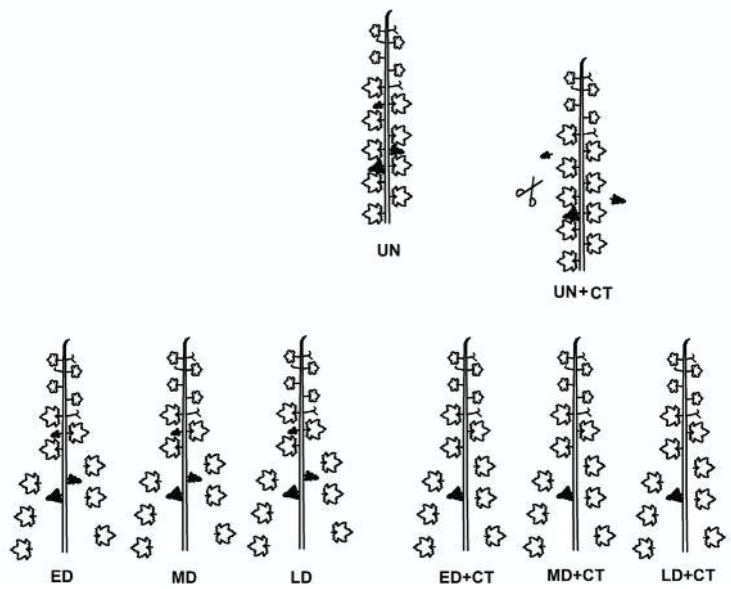

FIGURE 1. Treatments. Leaf removal (LR) was applied at three time points, corresponding to different phenological stages, denoted as ED (7 days after flowering), MD (30 days after flowering), and LD (at veraison). Cluster thinning (CT) was performed 7 days after flowering.

thinning was applied 7 days after flowering without LR. On the treated vines, LR treatment and CT were applied only once. A fully randomised block design was applied in the experiment. Each treatment included three replicates, with eight vines per replicate.

1. Analyses

Yield (kg/m^2) was determined at harvest by weighing all the grapes of each replicate. Average cluster weight (g) was obtained by weighing ten clusters per replicate. *Botrytis* incidence was determined as a percentage by visual assessment of the cluster health status (% of bunches infected) at harvest time. Berry weight (g) was determined in random samples of 30 berries per replicate. Then, these berries were collected in a plastic bag and stored in the freezer at - 20 °C until required for the analysis of the total anthocyanins, skin weight, weight of seeds and number of seeds. Moreover, total soluble solids (TSS) content in the juice (%) was detected using an Oechsle hydrometer after crushing all the grapes at harvest. Titratable acidity (g/L) of the juice was analysed by adding 10 % NaOH drop-by-drop until the acids were neutralised.

One month after the harvest, the frozen berries were taken for further analyses. The seeds were then separated, weighed and counted. The berry skins were separated, weighed and extracted in ethanol/water/hydrochloric acid (in the 70:29:1 v:v:v ratio) solution overnight for total anthocyanins analysis. Then, the absorbance value at 540 nm was read using a spectrophotometer and was converted to the malvidin-3-O-glucoside concentration values by multiplying the absorbance by 16.17 and by the dilution factor.

2. Microvinifications

From each replicate, grapes were destemmed, crushed, and 15 mg L^{-1} SO_2 was added before being inoculated with *Saccharomyces cerevisiae* (Uvaferm BDX). Fermentations were conducted in 5 L glass fermenters at a temperature of 25 °C. The pomace was mixed twice a day. After eight days of fermentation and maceration, the liquid phase (wine) was separated. Wines were racked twice, at 14 and 60 d after the end of fermentation. Then, the wines were bottled and stored at 12 °C. After 6 months, the samples were collected and stored at -20 °C until required for analysis.

3. High-performance liquid chromatography (HPLC) of anthocyanins

HPLC analyses included the wines produced in 2015, 3 months after the samples were frozen. Prior to HPLC analyses, wines were centrifuged for 3 min, and the supernatant was transferred into HPLC vials. Sample preparation was performed according to the OIV-MA-AS315-11 protocol (OIV, 2007). Anthocyanins were injected into an Agilent 1100/1200 series HPLC system equipped with an Agilent photodiode array detector (DAD). Separation was performed on a

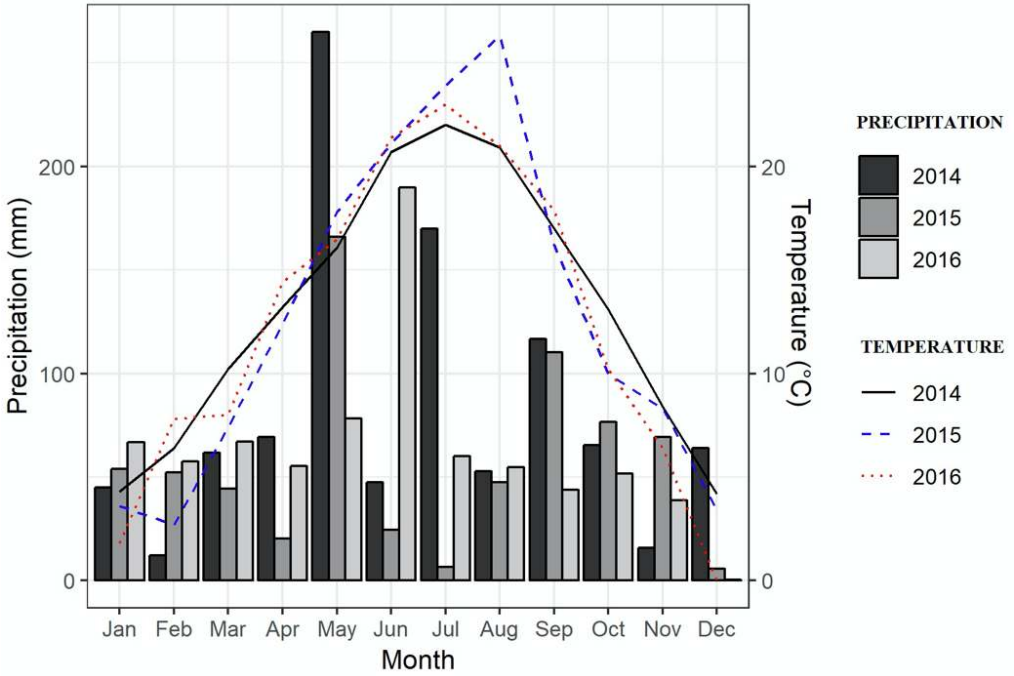

FIGURE 2. Average monthly precipitation and temperatures during the 2014-2016 period.

TABLE 1. Yield, cluster weight and incidence of *Botrytis* sp. for Cabernet-Sauvignon and Probus (2014-2016).

			Grape yield (kg/m^2)		Cluster weight (g)		*Botrytis* sp. (%)	
			Cabernet-Sauvignon	Probus	Cabernet-Sauvignon	Probus	Cabernet-Sauvignon	Probus
Treatment	Cluster thinning	CT	0.49	0.92	145	239	3.6	12.6
		No CT	0.89	1.46	136	206	4.3	11.6
	Leaf removal	ED	0.55^{b1}	1.16	123 ^{b}	209	3.4	9.5^{b}
		MD	0.72^{a}	1.18	144^{a}	230	4.1	12.5^{ab}
		LD	0.69^{a}	1.21	150^{a}	234	4.3	11.9^{ab}
		UN	0.80^{a}	1.20	146^{a}	217	4.1	14.3^{a}
Average		2014	0.40	0.78	98	170	10.2	20.0
		2015	0.80	1.39	147	266	1.7	5.8
		2016	0.87	1.39	177	230	0.0	10.3
		2014–2016	0.69	1.19	141	222	4.0	12.1
Statistical significance		CT	**	**	ns	**	ns	ns
		LR	**	ns	**	ns	ns	*
		Y	**	**	**	**	**	**
		CT × LR	ns	ns	ns	ns	ns	ns
		CT × Y	*	ns	ns	*	ns	ns
		LR × Y	ns	**	ns	ns	ns	ns
		CT × LR × Y	ns	*	ns	ns	ns	ns

Factorial ANOVA with three factors (CT, LR and Y). ^{a,b}indicate a significant difference among leaf removal factor levels. *p < 0.05. *p < 0.05, **p < 0.01, ns, nonsignificant.

reversed-phase column LiChrospher 100 RP 18 (5 µm) in LiChroCart 250-4 (MERCK) with a guard column LiChroCart 4 mm RP 18 (MERCK), at a temperature of 20 °C. The following HPLC-grade solvents were used: water/formic acid/acetonitrile (87:10:3, v:v:v) as solvent A, and water/formic acid/acetonitrile (40:10:50, v:v:v) as solvent B. Elution was performed at a flow rate of 0.4 ml/min, using a gradient elution, starting with 6 % (B), increasing to 30 % (B) after 15 min, 50 % (B) at 30 min, and 60 % (B) at 35 min, before decreasing to 6 % (B) at 41 min. The detection wavelength of 520 nm was utilised for all measurements. Anthocyanin compounds were identified by comparing the retention time with available standards, or the spectral characteristics with data published in the pertinent literature (Burns *et al.*, 2002; Ryan and Revilla, 2003; Radovanović and Radovanović, 2010). Anthocyanins were quantified using a seven-point external calibration curve (R2 = 0.9997) obtained by injecting standard solutions of

TABLE 2. Berry weight, skin weight number and weight of seeds per berry for Cabernet-Sauvignon and Probus (2014-2016).

		Berry weight (g)		Skin weight (g)		Number of seeds/berry		Weight of seeds/berry (g)	
		Cabernet-Sauvignon	Probus	Cabernet-Sauvignon	Probus	Cabernet-Sauvignon	Probus	Cabernet-Sauvignon	Probus
Cluster thinning	CT	1.38	1.76	0.41	0.68	1.9	1.9	0.08	0.09
	No CT	1.45	1.83	0.39	0.64	1.9	1.9	0.08	0.09
Treatment									
Leaf removal	ED	1.40	1.81	0.40	0.66	2.0	2.0	0.08	0.09
	MD	1.46	1.81	0.42	0.64	1.9	1.9	0.08	0.09
	LD	1.44	1.86	0.40	0.75	2.0	1.8	0.08	0.09
	UN	1.35	1.67	0.37	0.62	1.8	1.8	0.07	0.08
Average	2014	1.48	2.13	0.58	0.84	1.9	1.8	0.08	0.08
	2015	1.33	1.98	0.32	0.58	1.9	2.0	0.08	0.09
	2016	1.20	1.80	0.27	0.52	1.9	2.0	0.09	0.09
	2014–2016	1.41	1.79	0.40	0.66	1.9	1.9	0.08	0.09
Statistical significance	CT	ns	ns	ns	ns	ns	ns	ns	ns
	LR	ns	ns	ns	ns	ns	ns	ns	ns
	Y	**	**	**	**	ns	ns	ns	ns
	CT × LR	ns	ns	ns	ns	ns	ns	ns	ns
	CT × Y	ns	ns	ns	ns	ns	ns	ns	ns
	LR × Y	ns	ns	ns	ns	ns	ns	ns	ns
	CT × LR × Y	ns	ns	ns	ns	ns	ns	ns	ns

Factorial ANOVA with three factors (CT, LR and Y). *$p < 0.05$. **$p < 0.01$, ns, nonsignificant.

malvidin-3-monoglucoside chloride. All analyses were performed in triplicate and results were expressed as mean values.

Intraday repeatability and reproducibility were determined using an acidic ethanol-water extract ($EtOH/H_2O/HCl$, 70:29:1, v:v:v) of grape skins from the 'Pinot noir' cultivar (VIVC variety number 9279). Repeatability and reproducibility were expressed as relative standard deviations (RSD) of five anthocyanin monoglucosides (delphinidin, cyanidin, petunidin, peonidin, and malvidin). For intraday repeatability, the extract was injected into the HPLC system eight times within 24 h. Intraday variation was evaluated on five consecutive days.

4. Statistical analyses

Statistical analyses were performed using *R* software. The data was processed by multifactorial ANOVA. Duncan's test was used to test the significance of differences ($p < 0.05$) among the mean values of measured parameters. The normality of distribution was tested by using an Anderson-Darling test. When the data was not normally distributed, the nonparametric Kruskal-Wallis test was applied. Graphs were generated using the ggplot2 package.

RESULTS

Weather conditions for the experimental site during (2014-2016) are shown in Figure 2.

2014 was extremely rainy, especially in May and July, but 2015 and 2016 were dryer and hotter. In 2014, rainy weather caused berry cracking in Probus, which reduced yield and quality. Flowering occurred in the last 10 days of May in 2015 and 2016, and in the first 10 days of June, for 2014 in both varieties. Grapes were harvested in the first 10 days of October each year.

ED decreased the yield in Cabernet-Sauvignon (Table 1). Supplementary Table 1 shows the separation of the means, demonstrating that the lowest yield in both varieties was recorded in 2014, if the vines were subjected to CT. Depending on the year and variety, the yield was reduced by 45 % on average, if CT was applied.

ED significantly decreased the cluster weight of Cabernet-Sauvignon compared to other LR treatments, while no effect was observed in Probus. The year significantly affected cluster weight of Cabernet-Sauvignon, whereas interaction effect CT × Y was observed for Probus.

TABLE 3. TSS, TA and total anthocyanins of Cabernet-Sauvignon and Probus (2014-2016).

			TSS (%)		TA (g/L)		Total Anthocyanins (mg/L)	
			Cabernet-Sauvignon	Probus	Cabernet-Sauvignon	Probus	Cabernet-Sauvignon	Probus
Treatment	Cluster thinning	CT	21.3	21.0^{A}	7.1^{B}	7.1	701	1449
		No CT	21.5	19.5^{B}	7.4^{A}	6.6	674	1515
	Leaf removal	ED	21.5	20.8	7.0^{b}	6.1	765^{a}	1568
		MD	21.4	20.1	7.0^{b}	6.7	643^{b}	1423
		LD	21.5	20.2	7.3^{ab}	6.6	684^{ab}	1393
		UN	21.3	19.9	7.6^{a}	8.0	657^{b}	1537
	Average	2014	22.9	21.0	7.8	8.8	766	1422
		2015	19.9	18.9	5.9	5.2	631	1420
		2016	21.5	20.9	8.0	6.6	664	1602
		2014–2016	21.4	20.3	7.2	6.9	687	1481
Statistical significance		CT	ns	**	**	*	ns	ns
		LR	ns	ns	**	**	*	ns
		Y	**	**	**	**	**	ns
		CT × LR	ns	ns	ns	ns	ns	ns
		CT × Y	ns	ns	ns	**	ns	ns
		LR × Y	**	ns	ns	**	ns	**
		CT × LR × Y	ns	ns	ns	ns	ns	ns

Factorial ANOVA with three factors (CT, LR and Y).
[A,B]indicate a significant difference between CT and NoCT at $p < 0.05$.
[a,b]indicate a significant difference among leaf removal factor levels at $p < 0.05$.
*$p < 0.05$. *$p < 0.05$, **$p < 0.01$, ns, nonsignificant.

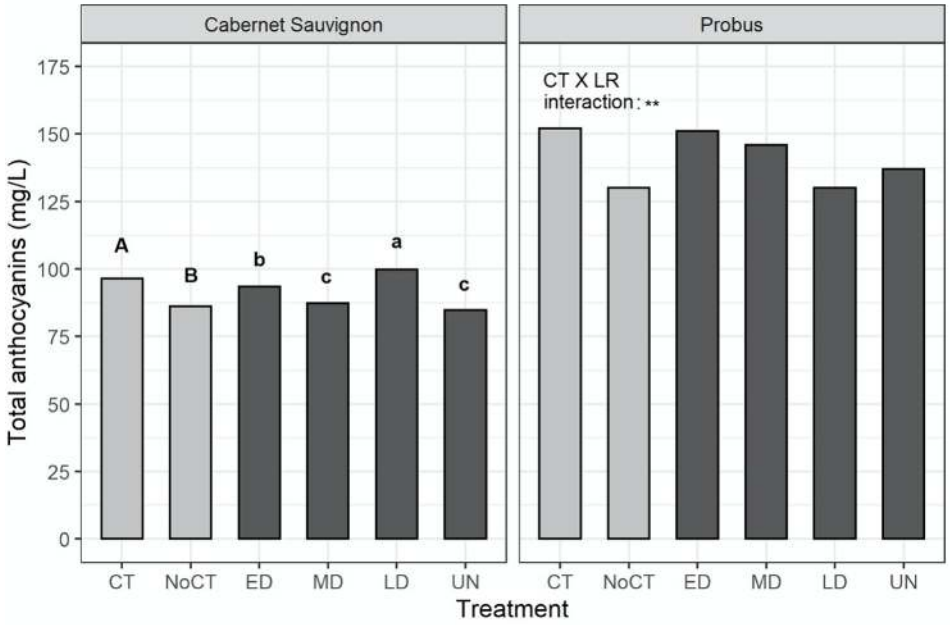

FIGURE 4. Tri-substituted/di-substituted and methoxylated/hydroxylated anthocyanins in the wines of Cabernet-Sauvignon and Probus (2015).
Factorial ANOVA with two factors (CT and LR).
[a,b,c]indicates a significant difference among LR factor levels at $p < 0.05$

The year significantly affected the incidence of *Botrytis* sp. in both varieties. Moreover, ED significantly reduced *Botrytis* sp. incidence in Probus compared to undefoliated vines.

The treatments had no effect on berry weight, skin weight, number and weight of seeds per berry (Table 2). However, differences in berry and skin weight across the years were observed in both varieties. Mean ± standard error for all parameters related to the grape quality across the years are shown in Supplementary Tables 2,3,4 and 5.

CT increased TSS content in Probus, but no effect was shown on Cabernet-Sauvignon (Table 3). Interaction LR × Y affected TSS content in Cabernet-Sauvignon, while Probus was unaffected by LR. CT and LR treatments decreased titratable acidity in Cabernet-Sauvignon.

Interactions CT × Y and LR × Y affected TA in Probus. The highest TA was recorded in 2014 in undefoliated vines (11.2), and the lowest was recorded in 2015 in ED treatment (4.8 g/L) (Supplementary Table 1). ED increased total anthocyanins in the grape skin compared to undefoliated vines. CT showed no effect on total anthocyanins in the grape skin of both varieties. Interaction LR × Y affected total anthocyanins in Probus (Supplementary Figure 1).

CT, ED and LD increased total anthocyanins in the wine of Cabernet-Sauvignon (Figure 3). For Probus, interaction CT × LR affected total anthocyanins. The highest content of the total anthocyanins (169.8 (mg/L)) was observed in Probus wine that received the treatment ED + CT (Supplementary Figure 1).

LD treatments decreased the tri-substituted anthocyanins content in Cabernet-Sauvignon wine compared to UN (Figure 4). In Probus, no effect was observed. In all treatments, di-substituted anthocyanins were present (up to 9 %) in the wines of both varieties. In the wines of Cabernet-Sauvignon, the lowest percentage of methoxylated anthocyanins was observed in ED treatment (90.9 %).

CT and LD increased acylated anthocyanins in Cabernet-Sauvignon, while in Probus no effect was shown (Figure 5). In the wines of Cabernet-Sauvignon and Probus, acylated anthocyanins were present at up to 27 and 24 %, respectively. In Cabernet-Sauvignon, MD and LD showed a lower percentage of coumaroylated anthocyanins, compared to undefoliated vines. In both varieties, monoglucoside percentage was unaffected by the treatments.

CT decreased the percentage of malvidin in Probus, while Cabernet-Sauvignon was unaffected (Figure 6). The varieties reacted differently to LR treatments: in Cabernet-Sauvignon, LR treatments ED and LD decreased the percentage of malvidin, whereas in Probus MD treatment increased it.

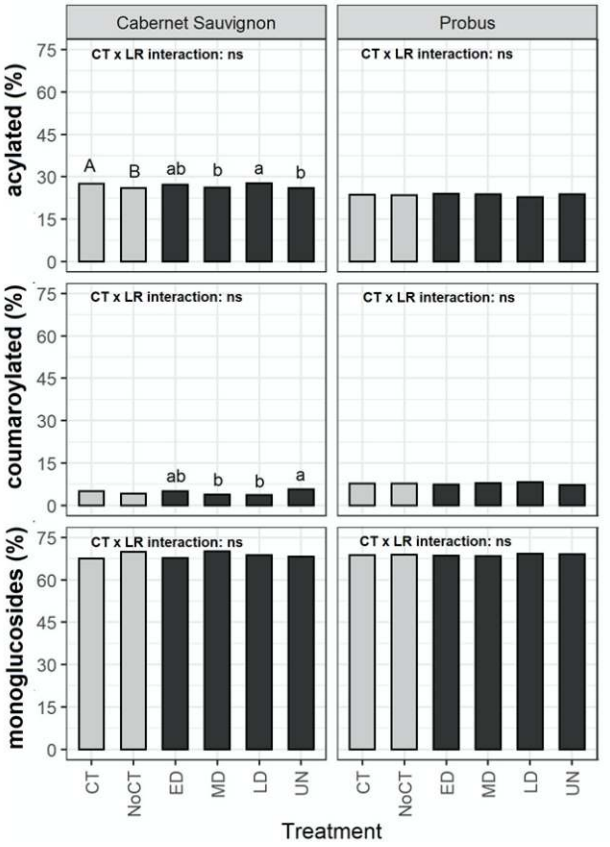

FIGURE 5. Percentages of acylated anthocyanins, coumaroylated anthocyanins and monoglucosides in the wines of Cabernet-Sauvignon and Probus (2015).

Factorial ANOVA with two factors (CT and LR). ^{A,B}indicates a significant difference between CT and NoCT at $p < 0.05$. ^{a,b}indicate a significant difference among LR factor levels at $p < 0.05$.

CT and ED increased the percentage of cyanidin in Cabernet-Sauvignon wine. Interaction CT× LR affected the percentage of cyanidin in Probus wine. The highest percentage of cyanidin was through ED + CT treatment, and the lowest was with through MD treatment (Supplementary Figure 1).

ED increased the percentage of Delphinidin in the Cabernet-Sauvignon wine, whereas Probus was unaffected. Peonidin was the most affected anthocyanin in Cabernet-Sauvignon after LR treatments wine, at a percentage more than twice that of the wine derived from undefoliated vines. In the wines of both varieties, treatments did not change the percentage of petunidin.

DISCUSSION

Our findings suggest that CT and LR improved grape quality and modified the anthocyanin ratios in wine. However, the varieties reacted differently to these treatments, as evident from the variation in grape quality. Moreover, year - either alone or in interaction with other factors - affected all tested parameters except seed number and weight.

CT was always conducted 7 days after flowering, as its timing had a limited effect on the grape and wine quality (King *et al.*, 2015). In the climate conditions of Serbia, it is advisable to perform this operation after flowering because of unpredictable weather conditions, which can adversely affect berry-set. Thus, an additional crop removal before berry-set would be undesirable.

ED affected grape yield differently depending on the variety. Tardaguila *et al.* (2010) reported similar results for Cabernet-Sauvignon to those we observed: they found that the yield was reduced by 30−70 % by early LR. Bešlic *et al.* (2013) investigated the effect of early LR on the yield parameters of Cabernet-Sauvignon and Prokupac, and observed that early LR decreased berry size and number of berries per cluster, which lowered the yield.

Moreover, berry cracking of Probus, in rainy 2014, increased the incidence of *Botrytis* sp., particularly in UN. Lower incidence of *Botrytis* in treatments involving LR could be related to better aeration of clusters and lower bunch compactness in ED. Lower incidence of *Botrytis* as a result of early LR treatment was also observed by Palliotti *et al.* (2012). However, as there was a high variation in *Botrytis* incidence among the plots subjected to the same treatment, in future research the incidence should be recorded for each vine separately.

Although the difference was not statistically significant, berry weight was higher in samples subjected to CT treatments. Gli Munoz *et al.* (2009) also observed that CT tends to increase berry weight of Tempranillo and Syrah.

TSS increased in Probus following CT treatments, while no effect on Cabernet-Sauvignon was observed. Probus has around 30 % heavier clusters compared to Cabernet-Sauvignon, which could be the reason for a different response to CT. In addition, Gil *et al.* (2009) reported a varietal behaviour responding to CT; while Tempranillo significantly increased TSS following CT, no effects where shown for Shiraz. High temperatures have been shown to contribute to lower TSS (Greer and Weston, 2010) and TA (Buttrose *et al.*, 1971; Brandt *et al.*, 2019). Karoglan *et al.* (2014) and Zhuang *et al.* (2014) observed that TSS content

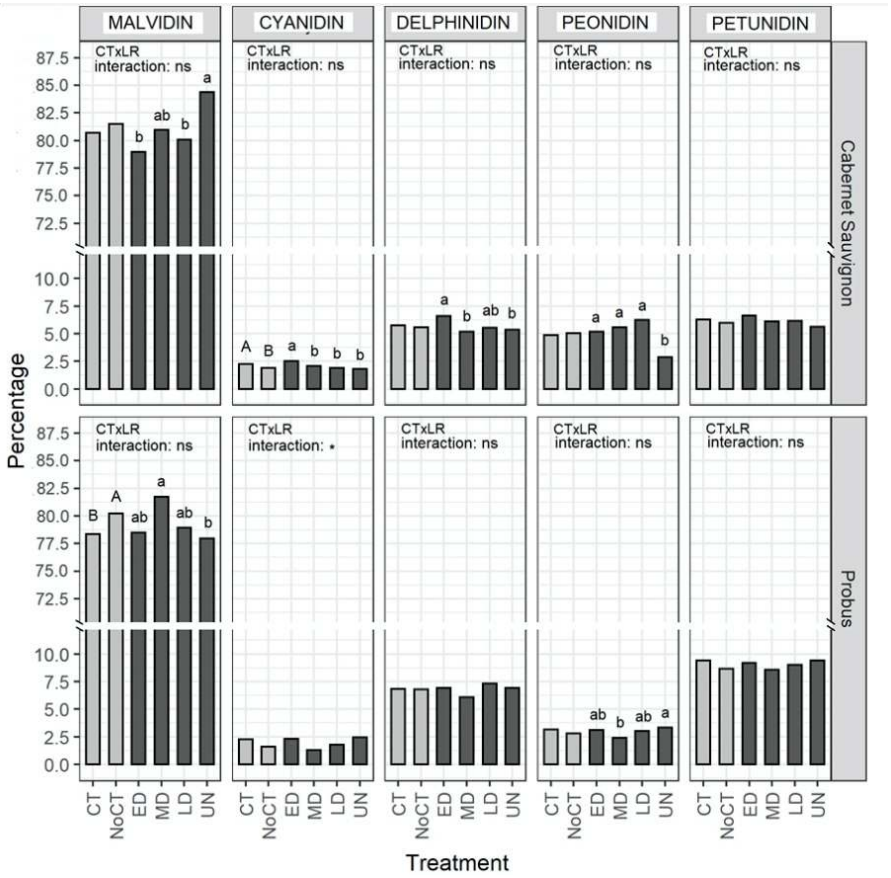

FIGURE 6. Percentages of individual anthocyanins and their derivates in the wines of Cabernet-Sauvignon and Probus (2015).

Factorial ANOVA with two factors (CT and LR). [A,B]indicates a significant difference between CT and NoCT at $p < 0.05$. [a,b]indicate a significant difference among LR factor levels at $p < 0.05$.*$p < 0.05$. *$p < 0.05$, **$p < 0.01$.

in the grape juice of red varieties was unaffected by CT.

The titratable acidity of cluster thinned Probus vines tended to be higher than in control vines, while in Cabernet-Sauvignon the opposite was true. di Profio *et al.* (2011) and Reščić *et al.* (2015) reported that CT decreased titratable acidity, while Valdes *et al.* (2009) failed to observe any effect.

LR usually reduces titratable acidity (Bledsoe *et al.*, 1988; Petrie and Clingeleffer, 2006) or has no effect (Kemp, 2010; Sivilotti *et al.*, 2016). In the present study, a decrease in titratable acidity was noted in Cabernet-Sauvignon, whereby Probus was affected by LR × year interaction. The effects of LR applied around veraison (onset of ripening) on grape quality were less consistent, possibly due to the competition in the accumulation of photoassimilates between fruits and roots, which starts around veraison (Morinaga *et al.*, 2003).

Poni *et al.* (2006) have shown that the increase in seasonal carbon supply per crop unit (up to 38 %) is the main factor behind the enhanced grape quality in defoliated vines compared to controls. These authors further noted that quality improvement can be attributed to a combination of lower yield, lower canopy age and photosynthesis compensation. Verdenal *et al.* (2017) observed that enhanced wine quality could be related to greater skin thickness following LR.

Surprisingly, the total anthocyanin content in the skin of Probus variety was also unaffected by year. These findings could be related to the higher difference in the berry composition within the Probus cluster compared to Cabernet-Sauvignon. In the present study, a higher standard error was noted for all tested Probus grape quality parameters.

The temperature during the growing season is directly related to grape maturity. Therefore, higher total anthocyanins in the skin and wine observed in our research could be the consequence of a temperature increase caused by LR. This is crucial to fulfilling thermal requirements needed for fruit maturation in cool summers (Frioni *et al.*, 2017).

Ristic *et al.* (2007) found that exclusion of sunlight from the cluster decreased total anthocyanins.

The results yielded by the present study indicate that LR increased peonidin and its derivatives in the Cabernet-Sauvignon wine and ED increased the hydroxylated anthocyanin content. It also affected hue and colour stability, which are influenced by the hydroxylation and methylation pattern of the B ring of the anthocyanidins (He *et al.*, 2010). An improvement in Cabernet-Sauvignon and Uni Blanc grape and wine quality as a result of the combined effects of CT and LR was also observed by Song *et al.* (2018). In the future, it would be interesting to explore the effects of LR and CT on berry skin thickness.

CONCLUSIONS

CT and LR affected grape and wine quality in both varieties. Among the LR treatments, ED was the most effective in both varieties. The anthocyanins content in Cabernet-Sauvignon wine was increased by CT and LR, but a lower yield in ED and CT did not compromise wine quality improvement. However, Probus was more influenced by weather conditions during the season. No negative effect of ED on grape and wine quality was observed. Moreover, ED decreased the incidence of *Botrytis* sp. in Probus, so ED treatment should be performed each year in the Probus vineyards to prevent the incidence of *Botrytis* sp.

Acknowledgements: The research was supported by The Ministry of Education, Science and Technological development of the Republic of Serbia and its project TR 31027. We are grateful to our enologist Siniša Ostojić for his support of this work.

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